Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis

Laura Kennedy1, Elizabeth Evans1, Chiung-Mei Chen1, Lyndsey Craven1, Peter J. Detloff2, Margaret Ennis1 and Peggy F. Shelbourne1,*

1Division of Molecular Genetics, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G11 6NU, UK and 2Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, 720 20th Street South, Birmingham, Alabama, USA

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Huntington disease is caused by the expansion of a CAG repeat encoding an extended glutamine tract in a protein called huntingtin. Although the mutant protein is widely expressed, the earliest and most striking neuropathological changes are observed in the striatum. Here we show dramatic mutation length increases (gains of up to 1000 CAG repeats) in human striatal cells early in the disease course, most likely before the onset of pathological cell loss. Studies of knock-in HD mouse models indicate that the size of the initial CAG repeat mutation may influence both onset and tissue-specific patterns of age-dependent, expansion-biased mutation length variability. Given that CAG repeat length strongly correlates with clinical severity, we suggest that somatic increases of mutation length may play a major role in the progressive nature and cell-selective aspects of both adult-onset and juvenile-onset HD pathogenesis and we discuss the implications of this interpretation of the data presented.

INTRODUCTION

Huntington disease (HD) is an inherited neurological disorder, characterised by progressive motor, psychiatric and cognitive disturbances. Although end-stage autopsy brain material displays extensive neuronal loss and gliosis, the earliest and most severe changes occur in the medium spiny neurons of the striatum (1). The HD mutation was identified in 1993 as the unstable expansion of a CAG repeat coding for an extended polyglutamine tract in a ubiquitously expressed protein called huntingtin (2). The length of the expanded CAG repeat stretch in the mutant HD gene is variable (always >36 repeat units) and has a critical influence on downstream pathology since longer polyglutamine tracts are associated with more severe, earlier onset disease (2). The mutation is unstable in ~80% of intergenerational transmissions, showing both increases and decreases in size, although increases are more common, particularly in the paternal germline (reviewed in 3). A number of studies have also demonstrated that mutant CAG repeat sizes vary both within and between somatic tissues of HD patients (4–11). The greatest size variability appears to occur within regions of the brain that show most neuropathological involvement (6,8,10,11). This somatic size mosaicism is most prominent in juvenile onset cases of HD (6,8) where it has been reported that a subset of cells in the cortex/striatum have mutant CAG tracts that are up to 13 repeats bigger than cells in relatively spared regions of the brain such as the cerebellum (6).

We have previously explored the idea that tissue-specific somatic mutation instability may contribute to patterns of tissue-specific pathology in HD by investigating mutation length profiles in tissues derived from a knock-in HD mouse model (12). Our results concurred with a number of other HD mouse studies demonstrating age-dependent mutation length variability which, with the exception of the cerebellum, is most pronounced in a number of brain regions (11,13–16). However, in contrast to the other studies, we employed sensitive small pool PCR (SP–PCR) methodologies (17,18) to reveal brain-region specific, expansion-biased mutation length profiles characterised by size changes (gains of up to 200 CAG repeats) that were much greater than previously documented. Here we have extended these studies and present data...
suggesting that mutation length variability in human HD brain tissue is also much greater than previously reported and that increased somatic mutation sizes may contribute to both the progressive nature and cell-selective aspects of human HD pathogenesis.

RESULTS

Temporal and spatial mutation length profiles in HD mouse brain

In order to explore the relationship between somatic mutation instability and neuropathological involvement in HD, we used SP–PCR analyses to investigate HD mouse brain tissue. These confirmed that general mutation profiles (levels of mutation length variability and magnitude of size changes) in different brain regions of heterozygous Hdh6/Q72 knock-in HD mice (19) carrying ~72 CAG repeats were consistent in the 2–6 animals tested at each time point (see Fig. 1 for representative data). Although length changes of >5 CAG repeats are barely detectable in the striatum, cortex, hippocampus and cerebellum at 3 months of age, there is clear evidence of mutation instability (size changes of 5–30 CAG repeats) by 9 months of age, particularly in the striatum and cortex. The expansion-biased changes in repeat length then continue to accumulate over time in all brain regions. However, the rate and/or magnitude of length changes appears to differ in the brain regions examined because data from 24-month-old animals show length variability and magnitude of size changes that are greatest in the striatum, intermediate in the cortex and hippocampus and lowest in the cerebellum. As we have shown previously (12), many (>80%) cells in the striatum of a 24-month-old HD mouse contain mutant repeat tracts that have increased in size (Fig. 1). A small number of these (0.5–1%) are >250 CAG repeats in length. Whilst <1% cells in other regions of the brain display significant increases in mutation length (e.g. a doubling in size to >150 CAG repeats), the majority have much smaller mutation length changes (up to 10 repeats), particularly in the cerebellum.

Progressively impaired motor performance in HD mice correlates with age-related, expansion-biased mutation length variability in HD mouse brain

As longer inherited CAG repeat lengths correlate with increased disease severity, we predict that the age-dependent, expansion-biased alterations in the brain mutation length profiles of HD mice will be accompanied by progressive impairment of a measurable functional outcome. To test this idea, we assessed the motor performance of HD mice over time. Rotarod testing of male HD mice (n = 8) and male wild type littermates (n = 8) was performed at 4, 12 and 18 months of age (Fig. 2). Motor testing was also performed on cohorts of female HD (n = 8) and female wild type (n = 8) mice (data not shown). A 3-way repeated measures ANOVA (genotype of mouse × age × rod speed) and post hoc tests applied to these data indicated that the rotarod performance of male and female HD mice at 4 months of age was significantly worse (F1,383 = 11.21, P < 0.001; F1,383 = 14.5, P < 0.0001, respectively) than wild type littermates, irrespective of rod speed and day of testing. Compared to wild type littermates, motor performance at 12 and 18 months of age was also impaired in the male HD mice (F1,359 = 71.75, P < 0.0001; F1,335 = 68.78, P < 0.0001, respectively) and female HD mice (F1,383 = 81.97, P < 0.0001; F1,383 = 59.39, P < 0.0001, respectively). A 3-way ANOVA (individual mouse × age × rod speed) and post hoc tests also showed that, in contrast to their wild type littermates, the rotarod performance of HD male and female mice significantly deteriorated with age (HD males F2,431 = 12.6, P < 0.0001; wild type males F2,575 = 1.05, P = 0.35; HD females F2,575 = 5.62, P < 0.005; wild type females F2,575 = 3.28, P = 0.04). Rotarod deficits of HD mice cannot be attributed to weight differences as the weights of the male and female HD cohorts at all time points and the gain of weight between two consecutive time points were not significantly different from those of their wild type littermates (data not shown).

![Figure 1](https://example.com/figure1.png)
Tissue-specific mutation length profiles are influenced by the size of the progenitor CAG repeat length

To exclude the possibility that the mutation length profiles revealed by SP–PCR are an artefact of the Hdh/Q72-80 HD mouse lines studied, we also investigated the independently derived Hdh(CAG)150 knock-in HD mouse line carrying a mutation of \( \sim 150 \) CAG repeats (20). SP–PCR analyses reveal mutation instability in all somatic tissues tested in two \( \sim 11 \)-month-old Hdh(CAG)150 mice (Fig. 3A). Data from Hdh(CAG)150 mice indicated high levels of mutation length variability in the striatum and cortex with lower levels in the cerebellum, broadly consistent with the Hdh/Q72-80 findings (Fig. 1). However, in contrast to the Hdh/Q72-80 data, the mutant CAG repeat tract in the Hdh(CAG)150 mice is also very unstable in the liver (Fig. 3B). The changes in repeat length observed in the Hdh(CAG)150 mice show a clear bias towards expansion in all tissues, although reductions of up to 70 CAG repeats were observed in the mutation lengths of 3–5% of liver cells.

Figure 2. Progressive motor deficits of HD mice. The rotarod performance of heterozygous Hdh6/Q72 mice (\( n = 8 \) males, black circles) and their wild type littersmates (\( n = 8 \) males, grey squares) at 4, 12 and 18 months of age. The mice were subjected to three trials of 1 min at each of eight different speeds ranging from 5 to 40 rpm. Motor performances of each group of animals are expressed as their mean latency to fall in the three tests (±SEM).

Figure 3. CAG repeat copy number of the progenitor mutation influences tissue-specific mutation length profiles. (A) Representative data from the SP–PCR mutation length profiles within striatum (St), cortex (Cx), cerebellum (Cb) and liver of heterozygous Hdh(CAG)150 mice at 4 and 11 months of age. (B) The mutation length profiles in the liver of young and old HD mice demonstrates that the smaller mutation in Hdh6/Q72 mice is much more stable that the larger mutation in Hdh(CAG)150 mice. Each lane contains the products from 1 to 10 cells worth of DNA from the tissue indicated. The numbers on the left hand side of the panels indicate the number of CAG repeats carried by the Hdh alleles amplified (mo = months).

Tissue derived from two 4-month-old Hdh(CAG)150 mice (Fig. 3A) revealed lower levels of mutation instability compared to the tissues derived from the \( \sim 11 \)-month-old animals. The age-dependence of the mutation length profiles concurs with Hdh/Q72-80 findings, although the size changes in 3-month-old Hdh/Q72-80 mouse brain (Fig. 1) are far less variable than those observed in Hdh(CAG)150 mice of a similar age (Fig. 3A). This suggests that mutation length profiles
are CAG repeat length-dependent, with larger initial (or progenitor) mutations resulting in earlier onset of mutation instability. The CAG repeat length of individual mutant alleles in the various tissues of Hdh(CAG)150 mice was determined (Fig. 4). Similar studies in Hdh/Q72-80 mice have indicated that the median mutation length in the striatum is significantly greater than the corresponding measures in both the cortex and the cerebellum (12). Although similar trends are apparent in the data generated from the Hdh(CAG)150 mice, statistical analyses are not as clear cut. The median mutation length in the cerebellum is significantly lower than the corresponding measure in the striatum (P < 0.01), cortex (P < 0.001) and liver (P < 0.0001). However, the median mutation lengths in the striatum and cortex are not significantly different (P = 0.44) and the median mutation length in the liver is significantly greater than the corresponding measure in the striatum (P < 0.01).

The striking mutation instability observed in the liver of ∼11-month-old Hdh(CAG)150 mice contrasts with Hdh/Q72-80 mouse liver findings, that show little length variability even at 24 months of age (Fig. 3B). When larger numbers of DNA molecules are amplified in the same reaction, a bimodal distribution of mutation length is clearly observed in 11-month-old Hdh(CAG)150 mouse liver (data not shown) and in the histogram representing mutation length distribution (Fig. 4). Overall, the Hdh(CAG)150 mouse findings suggest that progenitor CAG repeat length may influence tissue-specific variation in mutation length profiles and that longer mutations may lead to a more generalised, expansion-biased increase in mutation lengths throughout the mouse brain and periphery.

Mutation length profiles in human HD brain tissue

To investigate whether the mutation length profiles observed in HD mice reflect those in human HD tissue, we performed SP–PCR analyses on brain tissue from an individual (B1) with end-stage HD, who died 10 years after diagnosis (Fig. 5). Brain region-specific differences in the resulting mutation length profiles were observed. Mutation size changes appear to be expansion-biased and much more variable than previously reported. Although all of the brain regions tested display mutation instability, the most extensive length variability occurred in the cortical areas tested. A sample of ∼500 mutant alleles from each cortical region revealed that >50% of cells have mutation length changes of >5 CAG repeats and ∼25% of the mutation lengths have at least doubled in size (>170 CAG repeats). The largest mutation length detected was ∼700 CAG repeats, eight times the size of the progenitor allele (data not shown). Lower levels of mutation instability were observed in the hippocampus, substantia nigra and striatum.

On the face of it, these observations appear to contradict the prediction (based on the Hdh/Q72-80 mouse data presented) that the striatum would harbour the greatest mutation length variability. However, the pathological report indicated severe striatal atrophy and so cell loss may account for the mutation length profiles observed. Indeed, a hypothesis proposing that somatic mosaicism of mutation length plays a role in HD pathogenesis would predict that the cells with the largest mutations will disappear first.

In order to clarify this issue, we examined samples of brain tissue from individuals with a neuropathological classification of Vonsattel Grade 0, i.e. no microscopic evidence of pathological cell loss in the striatum (21). SP–PCR analyses of brain samples from individual A1 (inherited CAG repeat length = 41, age at death = 40 years) indicated high levels of mutation instability in the striatum but little evidence of instability in the cortex and hypothalamus (Fig. 6A). We estimate that the mutation lengths of 10–15% cells in this striatal sample have size increases of >20 CAG repeats and ∼2% cells have mutation lengths that exceed 200 CAG repeats.

The largest mutation length detected exceeds 1000 CAG repeats, 20 times the size of the 41 CAG repeat progenitor allele. Many of the length changes in the striatum are much greater than those observed in sperm derived from males with similar sized progenitor mutations (22,23). The mutation length profiles in brain regions of individual A2 (inherited CAG repeat length = 51, age at death = 27 years), showed high levels of mutation instability in the striatum and cortex but very little evidence that the mutation was unstable in the cerebellum (Fig. 6B). We estimate that 8–10% of cells in the striatal and cortical samples have mutation length increases of >20 CAG repeats and 1–2% cells have mutation length increases of >150 CAG repeats. Alleles containing >1000 CAG repeats were detected in both striatal and cortical samples (data not shown). It is not known whether individuals A1 and A2 were symptomatic at the time of death but, using a previously published algorithm (24), a predicted age of neurological onset
was calculated as 53.3 years for individual A1 (age at death = 40 years, ~13 years before predicted age at onset of symptoms) and 33.1 years for individual A2 (age at death = 27 years, ~6 years before predicted age at onset of symptoms). Thus, dramatic mutation length increases in vulnerable brain regions not only occur as an early event in HD pathogenesis, they may also precede the onset of symptoms.

DISCUSSION

Given the widespread expression of mutant huntingtin (2,3) it is unclear why medium spiny striatal neurons are particularly vulnerable to the disease process. Among the hypotheses put forward to account for this cell-selective neuropathology are a number based on cell-type specific processing or localisation of the mutant protein (25–28) or the consequences of abnormal interactions between mutant huntingtin and brain region-specific protein partners (reviewed in 29). In this study we have investigated an additional hypothesis proposing that tissue-specific differences in mutation length profiles may play a role in the cell-selective aspects of HD pathogenesis. We have confirmed previous data suggesting dramatic age-dependent, expansion-biased changes in mutation length occur in the striatum in a mouse model of HD (12) and extended these studies to demonstrate patterns of expansion-biased mutation instability within HD mouse brain that parallels the topography and temporal order of neuropathological involvement in human HD. In order to determine whether these brain-region specific changes in mutation length have functional consequences for the HD mice, we monitored their performance on the rotarod. Impaired motor performance was detected at 4 months of age when few somatic mutation size changes are evident in HD mouse brain, suggesting that, at least with an inherited mutation length of >70 CAG repeats, onset of motor deficits can occur in the absence of mutation length variability. However, a continuing decline in motor function over the following 14 months was accompanied by expansion-biased mutation length changes in various region of the brain. Whilst confirmation of a direct causal link between the progressive mutation instability and the decline in motor function requires a more sophisticated experimental design, it is nonetheless tempting to speculate that the brain region-specific, expansion-biased size changes of the CAG repeat may play a role in progressive aspects of HD pathology and consequent symptomatology.

SP-PCR studies revealed that expansion-biased, age-dependent changes in CAG repeat length also occur in somatic tissues of the Hdh(CAG)150 knock-in HD mouse line (20). As all mice in the study were maintained on similar genetic backgrounds the major difference between the Hdh(CAG)150 and Hdh/Q72-80 lines lies in the mutation length transmitted through the germline, ~150 and ~80 CAG repeats, respectively. Previous studies from our group (12) and others (30) have indicated that patterns of somatic mutation length changes are highly reproducible within and between age-matched individual animals derived from the same mouse line modelling triplet repeat instability. Comparing data from the Hdh(CAG)150 and Hdh/Q72-80 lines therefore suggests that the progenitor copy number of the CAG repeat mutation has an influence on two properties of the resulting somatic mutation length profiles. Firstly, the larger the size of the progenitor CAG repeat mutation, the earlier the onset of somatic mutation instability. Secondly, the larger the size of the progenitor CAG repeat mutation, the more blurred the boundaries that define the tissue-specific mutation length profiles become. This results in more generalised patterns of high-level repeat instability throughout the brain and periphery. This phenomenon may account for the age-dependent mutation length variability reported in many central nervous system (CNS) and non-CNS tissues of the R6 lines of HD mice that also carry a large mutation of >130 CAG repeats (13,15). We hypothesise that smaller mutation lengths may have pathological consequences that are more tissue-specific in nature, whereas longer mutation lengths could have more global cytotoxic consequences. Consistent with this point is the observation that the most advanced (Vonsattel grade 4) neuropathological changes are seen in juvenile HD, where neuronal loss can extend into regions such as the cerebellum, a region spared in adult-onset patients (31). It has also been noted previously that individuals with juvenile onset of the group of neurodegenerative disorders associated with expanded polyglutamine-encoding CAG repeats often experience sympto- 
m instead of commonly observed in adult-onset cases of the respective diseases (32,33). It was suggested that this phenotypic overlap may occur because large expansions override cell-specific patterns of pathology and lead to a more generalised cytotoxicity throughout the brain and perhaps the periphery.
The insight and implications of these comments are bought sharply into focus with the demonstration that mutation lengths can be highly variable in human HD brain. In each of the three HD cases presented, the mutation length profiles were derived from a small area of the designated brain region, although for individuals A1 and A2 (Fig. 6), three separate sampling sites within each tissue block tested gave consistent results. The small sample set makes it difficult to comment on the relative importance of inherited mutation length and disease stage on inter-individual differences in mutation length profiles and given these caveats, it may be premature to draw general conclusions from the findings at this time. Nonetheless, if the data presented (Figs 5 and 6) are representative of the wider picture, they support the idea that expansion-biased size changes in HD mutation length occur earlier in striatal cells than other regions of the brain. Once the mutation length reaches a critical threshold size, it may trigger the pathological process, perhaps as a result of the increased polyglutamine load or by activating a toxic property of mutant huntingtin that increases non-linearly with polyglutamine length. Whilst other regions of the brain (particularly the cortex) are also prone to age-dependent increases in mutation length and, presumably, similar downstream pathogenic effects, the observed patterns of mutation instability predict a temporal gradient of and spatial differences in pathology that may help explain the extensive extra-striatal cell loss observed in human HD brains at the end of a protracted disease course.

Alternatively, the greater levels of mutation length variability observed in cortical regions of end-stage HD brain (Fig. 5) may indicate that non-cell autonomous mechanisms could increasingly contribute to HD pathogenesis as the disease progresses. For example, it has been suggested that striatal cell loss may occur as a result of glutamate excitotoxicity (34) or brain-derived neurotrophic factor depletion (35) mediated by the corticostriatal projections, and these cytotoxic processes may be exacerbated by increasing mutation size. On this point, it is interesting to note that mutation sizes late in the disease course appear to parallel the aggregate load in human HD brain tissue, which is generally higher in cortex than striatum (36). However, detailed analyses of many more samples of human autopsy brain material will be required before the relationship between mutation length, aggregate formation and nature of pathological sequelae can be clarified.

Still further explanations for the mutation length profiles of mouse and human HD brain are possible. For example, the mutation length changes may be an epiphenomenon or a consequence (rather than a cause) of the pathological processes responsible for region-specific neuronal dysfunction and degeneration. However, two observations argue that increased mutation lengths are more likely to have a primary role in effecting pathological events. Firstly, significant levels of mutation instability are present in human and mouse HD brain material early in the disease course, before the onset of pathological cell loss and possibly symptoms (in the human cases, A1 and A2). Secondly, mice carrying long, non-pathogenic CAG-CTG repeat tracts show significant repeat length changes within brain and peripheral tissues (30), suggesting cellular pathology is not necessarily required for initiating and/or sustaining changes in repeat tract size.

Further lines of evidence support the hypothesis that somatic increases in mutation size may play a role in HD pathogenesis.
This hypothesis predicts that increased mutation lengths in genomic DNA are transcribed and translated into longer polyglutamine lengths within the resulting mutant huntingtin protein. Previous studies of human (8) and mouse (16) tissue have demonstrated variable glutamine tract lengths of mutant huntingtin in the striatum that become more prominent over time. So what is the nature of the downstream pathology and how are the increased mutation sizes generated? In the striatum, does the HD mutation in the medium spiny neurons increase in size and trigger a cell-autonomous pathogenic pathway? Alternatively, are the increased mutation lengths present in glia, where they could activate a pathological mechanism that acts in a non-cell autonomous manner, perhaps by blocking trophic support or providing a toxic environment for the neurons? Which of the cellular processes implicated in generating triplet repeat length instability (replication, transcription, DNA repair or recombination (reviewed in 37) is likely to account for the mutation size changes observed in this study? The answers to these questions will be clarified when it is possible to ask whether mutation instability resides in a particular cell type or types. The SP–PCR approach used in this study relies on amplifying dilutions of DNA made from bulk tissue composed of heterogeneous cell types. The bimodal distribution of mutation size detected within the Hdh(CAG)150 mouse liver (Fig. 4) may indicate that this tissue has at least two distinct populations of cells that display significantly different levels of mutation instability. Interestingly, similar bimodal patterns of repeat instability are observed in liver tissue of other mouse lines carrying large (~130–160) CAG repeat tracts, including the R6 mice (13) and mice that model unstable CTG-CAG repeat tracts (38). However, more precise correlation of mutation length variability with specific cell types will most likely depend on the development of methodologies based on techniques such as laser capture microdissection of immunostained tissue (39).

The expansion of repeated sequence tracts is associated with an increasing number of inherited diseases that includes fragile X syndrome, spinal and bulbar muscular atrophy (SBMA), myotonic dystrophy, dentatorubral-pallidoluysian atrophy (DRPLA) and several types of spinocerebellar ataxia (SCA 1, 2, 3, 6, 7, 8 and 12) (40). Whilst the pathological bases of these diseases vary, they share features including the inverse correlation between repeat length and age at disease onset as well as intergenerational repeat instability associated with clinical anticipation (earlier age at onset and more rapid disease progression in successive generations of a family). In addition, most are characterised by distinct, tissue-specific patterns of cell death and dysfunction, in spite of the fact that the respective disease gene is widely expressed. The influence and consequences of somatic mutation length variability has been considered in many of these diseases. In myotonic dystrophy type 1 there is strong evidence that somatic mutation size may contribute to both the tissue-selectivity and progressive nature of symptoms, with the demonstration that somatic mutation length changes are both expansion-biased and age-dependent (18) and that muscle (a primary site of pathology in this disease) has larger mutation sizes than blood (18,41). Although different mutation sizes have been observed within brain tissue derived from individuals with SCA1 (42), SCA3 (43), DRPLA (39,44) and SBMA (45), a direct link between mutation length changes and patterns of neuronal vulnerability is less clear. However, our findings highlight the value of using sensitive detection techniques such as SP–PCR (or single molecule PCR) to reveal the full spectrum of mutation sizes as well as the importance of investigating candidate brain regions before onset of pathological damage.

**MATERIALS AND METHODS**

**Human and mouse brain tissue**

Human HD postmortem brain tissue for this study came from a number of sources and was used with appropriate approval from the local ethics committee. Microscopic examination of the striatal tissue by the Harvard Brain Tissue Resource Center confirmed that the brains from the HD mutation-positive individuals A1 and A2 demonstrated Vonsattel grade 0 pathology (21). No Vonsattel grade was assigned to the tissue obtained from the individual B1, but extensive cell loss and profound striatal atrophy was noted in the autopsy report. The predicted age of neurological onset was calculated according to the equation Log(age) = ß + β(CAG repeat number), where ß = 6.15 (SE = 0.095) and ß = −0.053 (24).

Tissue samples were harvested from HdhQ72 and Hdh4/Q80 mice (19) (hereafter collectively referred to as HdhQ72-80 mice) and Hdh(CAG)150 mice (20) of various ages and frozen at −80°C prior to DNA extraction. All mice used in this study were maintained on a hybrid 129/Sv × C57BL/6 background.

**Preparation of genomic DNA and CAG repeat copy number determination**

DNA from all samples was prepared using the Nucleon ST DNA extraction kit (Tepnel Life Sciences, Manchester, UK), following the manufacturer’s instructions. The human HD CAG alleles were amplified from genomic DNA in a reaction mixture containing 10% DMSO, 1.2 μM LKH1 (5′-CCCCATT-CATTGCCCCCGGTGCTG-3′) and LKH5 (5′-TGGGTGTGCT-GGGTCATCTGTC-3′) primers, 1 × ABgene Custom PCR Master Mix (45 mM Tris–HCl, pH 8.8, 11 mM (NH4)2SO4, 4.5 mM MgCl2, 0.113 mg/ml BSA, 4.4 μM EDTA, 1.0 mM each of dATP, dCTP, dTTP and dGTP) (Advanced Biotechnologies Ltd, Surrey, UK) and 0.1 U/μl of Taq DNA polymerase (Sigma, Poole, UK). The reactions were subjected to the following PCR protocol: 94°C for 4 min followed by 30 cycles of 94°C for 45 s, 68°C for 45 s, 70°C for 3 min, followed by an annealing step of 68°C for 1 min and a final extension step of 70°C for 10 min. A 484 bp product is amplified from an unaffected HD locus with a repeat configuration of (CAG repeat number), where ß = 6.15 (SE = 0.095) and ß = −0.053 (24).

For small-pool PCR (SP–PCR) analyses, DNA was digested with HindIII and diluted in 10 mM Tris–HCl, pH 7.5, 1 mM EDTA and 0.1 μM carrier primer LKH1 to a final concentration of between 6 pg and 100 ng input DNA per reaction prior to amplification. The number of molecules amplified in a reaction was predicted using Poisson analysis. The PCR products were loaded on 1.5% agarose gels (40 cm long) and electrophoresed in 0.5 × TBE buffer at 180 V for ~18 h at 4°C. 200 ng of marker (1 Kb Plus or 1 Kb size ladders, Gibco-BRL, Paisley,
UK) was loaded in both outside lanes and one middle lane of the gel. Southern blot analysis was performed using a radiolabelled probe comprising ~20 ng of a small fragment containing ~80 CAG repeats and ~70 bp flanking mouse Hdh DNA (i.e. the amplicon defined by MHD4 and MHD5 primers) plus 800 pg of the size marker. The amplified HD CAG alleles were revealed by autoradiography and sized using Kodak (Newhaven, CT) 1D software. All PCRs were set up in a laminar flow hood. No contaminating alleles were detected in the ~20% of PCRs per run that were zero DNA controls.

Genotyping and SP–PCR analysis of Hdh/Q72-80 and Hdh(CAG)150 mouse tissue using MHD16 and MHD18 primers was performed as previously described (12). The statistical significance of CAG mutation length comparisons in different tissues was calculated using the Mann–Whitney U-test.

Measuring motor performance of HD mice

HD and wild-type mice were the progeny of an N6 backcross of the Hdh6/Q72 line to the C57BL/6 inbred strain. Motor performance was measured between 10 am and 3 pm, using a rotarod apparatus comprising a rotating rod (diameter 3.5 cm) placed 60 cm above the apparatus floor and individual compartments for each mouse. Briefly, each mouse was subjected to the same 10 day procedure at 4 months of age. The first day was used to acclimatise the mouse to the apparatus (1 min trial at each of 0, 5, 10, 15, 20 rpm). The following 3 days were used to train the mice (three 1 min trials at 20 rpm each day). Testing was performed on days 8–10 with 1 trial at 5, 10, 15, 20, 25, 30, 35 and 40 rpm on each of the three days. The length of time that each animal was able to stay on the rod at each rotation speed was recorded. During the week prior to testing at 12 and 18 months of age, each mouse was retrained by subjecting it to 3 trials a day at 20 rpm (maximum 1 min) on 2 consecutive days. During all rotarod training and testing, mice were allowed a 5 min rest between two consecutive trials to reduce the effects of stress and fatigue.

Analysis of variance (ANOVA) and Tukey HSD post hoc tests were used to evaluate the data. Effects were considered significant when $P < 0.05$. Computations were performed in MINITAB (Minitab Inc., PA, USA).

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